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ENGINEERING HYDROGELS FOR DELIVERY OF THERAPEUTIC PROTEINS

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

BACHELOR OF SCIENCE
IN
BIOENGINEERING

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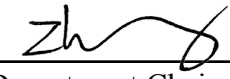

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Department Chair — date

ENGINEERING HYDROGELS FOR DELIVERY OF THERAPEUTIC PROTEINS

By

Francesca Briggs, Daryn Browne

SENIOR DESIGN PROJECT REPORT

Submitted to
the Department of Bioengineering

of

SANTA CLARA UNIVERSITY

in Partial Fulfillment of the Requirements
for the degree of
Bachelor of Science in Bioengineering

Santa Clara, California

2022

Abstract

In this project, we investigate how innate hydrogel properties can be leveraged for controlled protein drug release platforms. Therapeutic proteins have many valuable applications within the medical field, however, professionals often face many obstacles with obtaining controlled drug release. This paper analyzes how the manipulation of hydrogel properties can improve protein drug release rates. We started these investigations by varying hydrogel concentrations since we saw that this affects the release of small molecules. Additionally, we wanted to see what the addition of a second hydrogel network would do to protein release rates. These experiments concluded that raising polymer concentrations and adding secondary polymers to hydrogel drug delivery systems resulted in more linear and controlled protein release profiles.

Keywords: hydrogels, protein release, drug delivery, therapeutic proteins, alginate, chitosan

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1. Introduction

1.1 Background and Motivation - Drug Delivery

Proteins are diverse macromolecules with a multitude of uses within different fields. Since they are essential within the human body, proteins have had many important applications within medicine and the healthcare field. There are plenty of types of remedial proteins like monoclonal antibodies, enzymes, hormones, etc, all with different mechanisms and the ability to treat different classes of diseases (de la Fuente et al., 2021). Ultimately, the pharmaceutical industry invests vast amounts of resources into the research and development of therapeutic proteins because of their great potential and promising applications in medicine. One obstacle that professionals face with protein-based therapeutics is the struggle to obtain controlled drug release profiles (Dimitrov, 2012, Franco et al., 2020). This problem is commonly faced with any class of therapeutic drug delivery, not just remedial proteins.

One potential solution to these difficulties within protein therapeutics is using hydrogels as a drug delivery platform. Hydrogels are water-based polymers with a variety of different applications within the bioengineering field. They serve purposes in tissue engineering, cosmetics, wound dressings, and plenty of other areas. Hydrogels are especially valuable in drug delivery because they can be manipulated to facilitate a variety of different drug delivery methods. They are extremely versatile with tunable properties to sustain the delivery of plenty of different classes of drugs and delivery techniques. Hydrogel therapeutics offer the possibility of a new innovative drug delivery platform that eliminates risks and complications that past approaches have often run into. They are biocompatible, non-toxic, easily accessible, cheap, and simple to use but highly effective in a number of medical treatments (Li et al., 2016). Through our Senior Design project, we investigated how hydrogels could potentially not only improve the efficiency of protein drug delivery mechanisms and rates but also how hydrogels could make the delivery of therapeutic proteins non-invasive yet still effective.

1.2 Literature Review

1.2.1 Protein Therapeutics

Proteins are the most abundant biological compounds in the cells that act as enzymes, hormones, structural elements, and antibodies. Many therapeutic proteins have been used to treat a wide variety of diseases including type 2 diabetes, multiple sclerosis, and high blood pressure. Throughout the research we read, we found that many therapeutic peptides and proteins have challenges when it comes to traveling across the cells in the body due to their large size and poor cellular penetration. Therefore, drug delivery systems with a carrier that could transfer these large molecules into cells are necessary for successful and efficient drug delivery. We constantly see uncontrolled drug release profiles with these types of medications since they often are delivered in repeated doses. This gives many opportunities for drug concentrations within the body to fluctuate above and below the optimal therapeutic range. As the pharmaceutical industry

continues to pursue protein drug-based therapies, professionals need to also discover a way to prevent drug concentrations from rising above the maximum safe concentration, where these treatments become toxic, and below the minimum effective concentration where the treatment is ineffective (Franco et al., 2020).

1.2.2 Nonlinear Release Profiles

Currently burst release and nonlinear drug release profiles are challenges within drug delivery, both for small molecule drugs and for therapeutic proteins. A burst release is characterized by a sudden uncontrolled release of the encapsulated drug shortly after the initial introduction into the body or a buffer solution. In an *in vivo* setting, this could lead to toxic effects as the dosage may rise above the maximum safe concentration (Yoo et al., 2020). When burst releases occur, the drug is released before the system is stabilized causing more complications and a lack of overall control. Burst releases go hand in hand with nonlinear release profiles, which were described previously. A nonlinear release profile indicates extremely ineffective delivery of the encapsulated protein therapeutic, whether that means too much of the drug is released, such as with burst release, or not enough of the drug is released.

1.2.3 Hydrogels for Drug Delivery

Through our research, we found various methods for approaching these challenges. We found hydrogels in particular to be the most promising biomaterial for the delivery of protein drugs. Hydrogels have shown great promise in therapeutic protein drug delivery research. Hydrogels are a material often used in drug delivery research since they are 90% water and highly porous so they can accommodate drugs for delivery and facilitate controlled release. Hydrogels are a useful biomaterial for drug delivery applications due to their unique characteristics such as their inherent biocompatibility, tunable properties, easy accessibility, low cost, and diversity of both natural and synthetic material options. This versatility allows hydrogels to contribute to many different modes of drug delivery in the form of oral capsules, injectable hydrogels, transdermal patches, microneedles, and many other platforms. Also, they can be further modified to sustain different pore sizes in order to support the delivery of different groups of drug molecules (Li, 2016). The use of hydrogels for drug delivery has been researched and the biomaterial shows promising results. Moreover, the manipulation of hydrogels can easily introduce desired properties like stimuli responsiveness, enhanced mechanical properties, or biocompatibility to standard hydrogel systems allowing them to be used in a vast variety of different therapeutic applications.

1.2.4 Alginate Hydrogels

In our literature review, we found that the most commonly used polysaccharides in recent hydrogel research aimed at protein delivery are alginate, hyaluronic acid, chitosan, and dextran (Censi, 2012). We began our specific research into the development of a therapeutic protein delivery system that utilizes the mechanical and chemical properties of hydrogels by looking at

alginate as the primary polymer for our system. Alginate is a low-cost marine material extracted from the cell walls of brown seaweed that is used in various biomedical applications as a natural polymer. In nature, alginates occur as calcium, magnesium, and sodium salts. These compounds are very important as they can form hydrogels through ion-crosslinking, such as Ca^{2+}-crosslinking. Strontium and barium ions were also identified as promoting crosslinking in alginate (George 2006). The G-block length and molecular weight affect the physical and mechanical properties of alginates and their resultant hydrogels (Kulkarni, 2012). Alginate proved to be low cost as well as versatile with many sought-after qualities. The three properties of alginate hydrogel systems that we varied were alginate concentration, the presence of a crosslinker, and the addition of a secondary hydrogel. As aforementioned, literature review identified calcium ions as a suitable crosslinker for alginate hydrogel beads so we moved forward with calcium chloride solution as a crosslinking agent (Omer, 2016, Vanderberg, 2017) As noted before, double network hydrogels offer the opportunity to introduce desirable features to hydrogel systems.

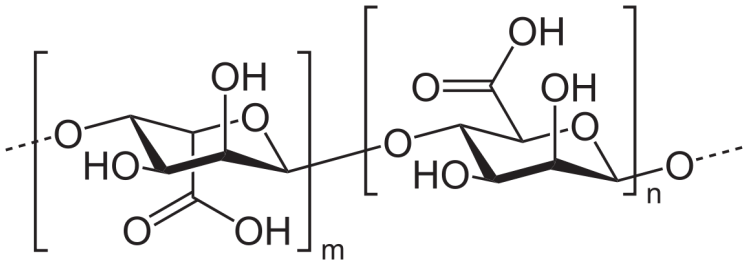

Figure 1. Chemical structure of alginate. Obtained from Wikipedia.

1.2.5 Chitosan Hydrogels

Chitosan is a polysaccharide that is derived from chitin, which composes the exoskeletons and endoskeletons of insects and crustaceans. This particular biomaterial has many applications in tissue engineering, wound dressing, and drug delivery of many different classes of medications. Like alginate, chitosan is non-toxic, biodegradable, and biocompatible, but also mucoadhesive and has evidence of containing pH and thermosensitivity (Peers, 2020). The structure of chitosan allows it to undergo many chemical modifications to suit many different means of drug delivery and be tailored for different classes of drugs. For example, chitosan hydrogels can reduce electrical resistance and aid in the encapsulation of larger macromolecules like protein drugs. Its cationic structure at a neutral pH also proves very resourceful in multiple applications (Khan, 2021). Unlike alginate, our literature review did not specify calcium 2+ ions as a suitable crosslinking agent for chitosan. Instead, we used sodium tripolyphosphate (TPP) solution as a secondary crosslinker for our double network hydrogels (Buranachai, 2010, Sun, 2011).

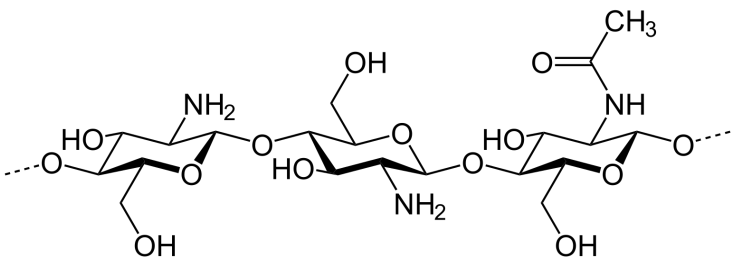

Figure 2. Chemical structure of chitosan. Obtained from Wikipedia.

1.2.6 Double Network Hydrogels

Double network (DN) hydrogels are a unique biomaterial in that they possess the polymer networks of two different hydrogels. DN hydrogels are formed by crosslinking two individual hydrogels. This is often done to supplement the primary hydrogel as when it is crosslinked with another polymer, the system takes on the properties of both networks. Through our research, we found that alginate-chitosan DN hydrogels are promising for a variety of applications including drug delivery and tissue engineering. In addition to having the desirable qualities of hydrogels such as biocompatibility and easily tunable properties, DN hydrogels also have higher mechanical strength and toughness due to the support of the second polymer network (Gu et al., 2018). Currently, there are limited studies that have been done on double network hydrogels, particularly within drug delivery.

1.2.7 Stimuli-Responsive Hydrogels

An additional property of hydrogels, such as chitosan, is stimuli-responsiveness. Stimuli-responsive hydrogels are especially advantageous for drug delivery platforms as they can be exploited to undergo physiological changes to achieve site-specific, controlled release of protein, peptide, and chemotherapeutic molecules for both local and systemic treatment applications (Sharpe et al., 2014). This would allow for more controlled delivery of protein drugs to a broader range of locations within the body. According to Dootsmohammadi et al, stimuli-responsive hydrogels have shown significant development in the targeted delivery of peptide hormones because of their natural characteristics such as networks, pore sizes, sustainability, and response to external stimuli (Doostmohammadi et al., 2019). For example, a pH-responsive hydrogel delivery system would allow for more efficient drug delivery to places that undergo extreme changes in pHs like the GI tract. Stimuli-responsive hydrogels offer another potential method for further controlling drug release in hydrogel-based drug delivery systems.

1.2.8 Micro-BCA assay

This project utilized a bicinchoninic acid (BCA) assay in order to quantify how much protein was released from the hydrogels over time. This is a colorimetric protein assay that is well established and commercially available. Within a sample, the reduction of Cu^{2+} ions to Cu^{+}

ions indicates the presence of a protein. These Cu^{+} ions are then able to bind to the BCA reagent, which produces a color change from blue to purple (Rogatsky, 2021). A greater protein concentration constitutes a more vibrant color change since more Cu^{+} ions are formed and available to bind. We utilized a microscale BCA assay because we expected our experiments to use less than 2 mg of protein. Normal BCA assays can measure from 2 mg to 10 mg of protein within samples, which would overall not be useful. Some other methods of measuring protein concentration include the Bradford protein assay and gravimetric analysis (Khramtsov, 2021). We chose the microscale BCA assay because of its specificity and simplicity compared to other methods.

1.2.9 Other Approaches

Hydrogels are not the only solutions that bioengineers and healthcare professionals are considering for controlled protein drug release. Some newer innovations in this field include nanoparticle, peptide, and liposome carrier drug delivery systems. Neither of these methods has been perfected and they often come with complications that can potentially be eliminated by a hydrogel-based delivery platform. Hydrogel systems provide the opportunity to provide more consistent drug release profiles and learn more about drug release mechanisms. Moreover, both of these alternative delivery methods also bring their own unique complications that hydrogels could potentially overcome. For example, nanocarrier systems tend to have issues with absorption in the GI tract due to the presence of mucosa (Bruno 2013). Furthermore, these nanoparticle systems also have to be larger in order to sustain protein drugs, leading to higher accumulation but restricted penetration of membranes (Mikyung 2015). Overall there are barriers limiting the absorption of nanoparticles, especially with oral drug delivery, that limit their application. On the other hand, peptide systems also are more susceptible to acidic degradation of drugs in low pH environments. This results in low drug availability at certain delivery sites like the colon and GI tract (Mikyung 2015, Patel 2015). Many hydrogel systems forgo this problem by using pH-resistant and responsive hydrogels that maintain their structure and stability in harsh environments. Furthermore, peptide carriers often struggle with poor permeability to membranes resulting in less efficient drug delivery. Both nanocarrier systems and peptide delivery systems share some obstacles like burst releases and nonlinear release profiles (Naeem 2020, Patel 2015). All in all, the tunable properties of hydrogels present the opportunity to eliminate these complications and more efficiently deliver protein drugs.

1.3 Team Management and Funding

Our team shared equal responsibility in designing and performing our experiments, collecting samples, and maintaining hydrogels. Dr. Asuri assisted us with the collection and analysis of our data. He provided ample guidance and input through our weekly meetings and email correspondence. Funding for our project was provided by Santa Clara University, School of Engineering. Our budget (Table 4) can be found in the Appendix.

1.4 Research Timeline

Due to the ongoing COVID-19 epidemic, the timeline of our experiment was reduced due to the closure of the labs. Additionally, some complications with funding requests also prevented us from ordering materials and beginning experiments as early as intended. We spent about five months planning our project and collecting information about hydrogels and hydrogel-based drug delivery platforms through our literature review. Additionally, during this time we were working on our independent study project which helped to inform this project. In February 2022, we began running our experiments in the lab and we finished our experiments in June 2022.

2. Project Goals and Restraints

Through our senior design project, we aim to investigate how hydrogel properties may be manipulated to create tunable release systems for delivering therapeutic proteins. First, we will vary the concentration of our hydrogels to see how polymer concentration impacts protein release rates. Then we will investigate how increasing the mechanical strength of our hydrogels impacts protein release rates and profiles by adding a second hydrogel network to our protein delivery platform. Changing the properties of hydrogels by adding secondary polymers, cross-linking hydrogels, or by increasing polymer concentration may change the effectiveness of the material in drug therapy. Overall, we would be establishing new methods for the controlled release of protein therapeutics.

2.1 Motivation - Small Molecule Delivery

In the summer and fall of last year, we took part in an independent study in which we examined the release of small molecule drugs from polyacrylamide, or pAAm, hydrogels. In this study, we used pAAm hydrogels as they are well characterized, commercially available, and routinely used in a wide variety of applications, including drug delivery. This research helped us learn how hydrogels could be manipulated to deliver small molecule drugs through various experiments, including varying polymer concentrations, introducing a second polymer network, and the use of nanoparticles. In particular, this study helped us understand that polymer concentration can be highly effective in changing release rates (Figure 3). These experiments indicated a strong correlation between polymer concentration and drug release as we can see that drug release rates were significantly reduced with increasing polymer concentration.

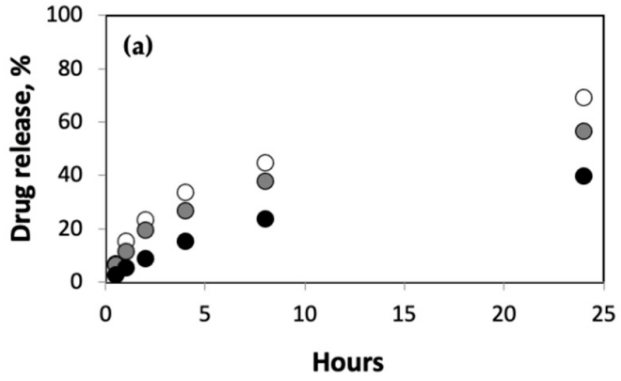

Figure 3. Drug release profiles of pAAm hydrogels. a. Drug release of 5-FU from 2.5% (white circles), 5% (grey circles), and 10% (black circles) pAAm hydrogels.

While this study was useful for understanding the tunable properties of hydrogels, much of the system design had to change due to differences in size between small molecule drugs and therapeutic proteins. For example, we were not able to use polyacrylamide hydrogels for these experiments because pAAm hydrogel pores are too small for protein molecules to pass through.

However, alginate's pores are larger and big enough to accommodate protein molecules. There are characteristics other than size that we needed to consider when applying our small molecule research to our protein release experiments. Previous studies have shown that protein drugs maintain, "high specificity and activity at relatively low concentrations, in comparison to small molecule drugs." This makes maintaining the physical and chemical stability of protein therapeutics more difficult (Ke Wang, 2010). Other considerations that may affect the stability of protein drugs are denaturation, aggregation, and hydrolyzation (Sariyer, 2020). Ultimately there are many factors and environmental conditions that can disrupt protein structure, stability, and activity. Throughout our design process, we had to keep these key differences in mind when applying our knowledge of small molecule delivery to the delivery of protein drugs.

2.2 Restraints

Throughout this project, we were not able to investigate how adjusting the crosslinking density of our polymer affected protein drug release rates. The reaction between alginate and calcium ions occurred instantaneously so we unfortunately were not able to examine crosslinking density as a variable with this system.We also ran into issues with the concentration of chitosan for our DN hydrogel tests. For these experiments, we wanted to use a higher concentration of chitosan, however, we were limited by the viscosity of our chitosan solutions. At higher concentrations, chitosan solidified and became a gel-like material that we could not pipette for our beads so we lowered the concentration.

3. Project Design: Materials and Methods

3.1 Materials

Materials for the preparation of the hydrogels, sodium alginate, chitosan (low molecular weight), and sodium tripolyphosphate (TPP) were purchased from Sigma Aldrich and calcium chloride and acetic acid were obtained from Santa Clara University. The bicinchoninic acid (BCA) protein assay was purchased from Sigma Aldrich and included bovine serum albumin (BSA) standard (3x1 mL, 2mg/mL), BCA solution (500 mL, bicinchoninic acid, sodium carbonate, sodium tartrate, and sodium bicarbonate in 0.1 M NaOH, pH 11.25), and 4% cupric sulfate. Horseradish peroxidase for the activity and viability studies was purchased from Sigma Aldrich.

3.2 Preparation of Hydrogel Samples

3.2.1 Alginate Hydrogels

All hydrogel samples for alginate bead protein release studies were prepared using stock sodium alginate solution (4% w/v), 100 mmol calcium chloride, and BSA standard (2 mg/mL). The alginate stock solution was diluted with DI water to the desired concentrations (3% and 1% w/v). The alginate solutions were mixed with the BSA standard in a 90:10 µl ratio and then added dropwise to 1 mL of the calcium chloride solution. The beads were left to crosslink for 10 minutes and then all the calcium chloride was removed and replaced with 1 mL of a buffer solution. We then removed a sample from each experiment every day for 5 days and measured the amount of protein released by our hydrogels using the micro-BCA assay.

3.2.2 Double Network (Alginate-Chitosan) Hydrogels

For our double-network hydrogels composed of alginate and chitosan, the beads were prepared using stock sodium alginate solution (4% w/v), stock chitosan solution (2% w/v), 100 mmol calcium chloride, sodium tripolyphosphate (TPP), and BSA standard (2 mg/mL). To prepare the hydrogel beads, we mixed the protein, alginate, and chitosan stocks in the appropriate ratios to obtain 200 µg/mL BSA and our desired alginate (1% and 2% w/v) and chitosan (0.4% and 0.8% w/v) concentrations. These solutions were then added dropwise to the calcium chloride solution and left for 10 minutes to allow the alginate to chemically crosslink. The calcium chloride was removed and then replaced with TPP and left to sit for another 10 minutes to chemically crosslink the chitosan. Finally, the TPP was removed and replaced with 1 mL of buffer solution. We then removed a sample from each experiment every day for 5 days and measured the amount of protein released by our hydrogels using the micro-BCA assay.

3.3 Analysis of Protein Release Rates

All of the protein release studies were analyzed using a standard BCA assay. We decided to run a micro-BCA assay since we only have 3 mL of the standard BSA solution provided in the BCA assay kit. First, we performed the serial dilutions as described in Table 1. Then, we made

the BCA standard reagent following the volumes listed in Table 2. We then ran a sample micro-BCA assay using the procedure provided with the BCA protein assay kit.

Table 1. BSA sample serial dilutions for a standard BCA assay

Dilutions for standard assay			
Tube	**Volume of BSA**	**Volume of diluent**	**Final BSA concentration**
1	250 µl from 2 mg/ml solution	250 µl	1,000 µg/ml
2	250 µl from tube 1	250 µl	500 µg/ml
3	250 µl from tube 2	250 µl	250 µg/ml
4	300 µl from tube 3	300 µl	125 µg/ml
5	100 µl from tube 4	400 µl	25 µg/ml
6	0	400 µl	0 µg/ml

Table 2. BCA standard reagent protocol for micro-BCA assay

Micro-scale assay		
BCA Solution	200 µl	4 ml
4% Cupric Sulfate	4 µl	80 µl

3.4 Statistical Analysis

Average and standard error was calculated using Microsoft Excel (v. 16.54) and the standard error was presented in the form of error bars in the graphs.

4. Results

4.1 Influence of Polymer Concentration on Protein Release Rates

First, we investigated the role of polymer concentration in protein release rates for different concentrations of alginate (Figure 4). These experiments show reduced protein release rates with increasing alginate concentration. It is important to note that the differences between protein release rates are significant; after 4 days, approximately 40% of the encapsulated protein had been released from the 3% alginate beads, while the 1% alginate beads had released >80% of the BSA. Additionally, the release profile (slope) of the 1% alginate hydrogels is consistent with burst release profiles, whereas the release profile for the 3% alginate hydrogels is more linear, indicating a more controlled protein release with increasing polymer concentration.

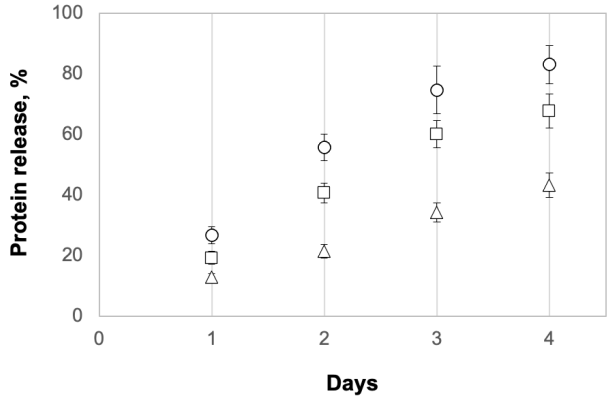

Figure 4: Protein release from alginate hydrogels at varying concentrations. Protein release (BSA) from 1% (circles), 2% (squares), and 3% (triangles) alginate beads. Data shown are the mean and standard error of triplicate measurements.

4.2 Protein Release Rates in Double-Network Hydrogels

To further investigate the tunable properties of hydrogels for protein drug release, we examined the effect of adding a second polymer network to our system. In these experiments, we crosslinked alginate and chitosan hydrogels to form an alginate-chitosan double network (DN) hydrogel. In our independent study research, we investigated DN hydrogels for the delivery of small-molecule drugs. We were interested to see if those observations could be extended to therapeutic protein delivery applications. Figure 5 compares the protein release rates of our hydrogels with varying concentrations of both alginate and chitosan. Figure 5a shows the release rates for our 1% alginate hydrogels crosslinked with chitosan while Figure 5 b shows the release rates of our 2% alginate hydrogels crosslinked with chitosan. These results confirm our results from the previous experiments indicating that polymer concentration reduces protein release rates. Furthermore, these results show that the use of DN hydrogels is another method with which we can control protein delivery rates.

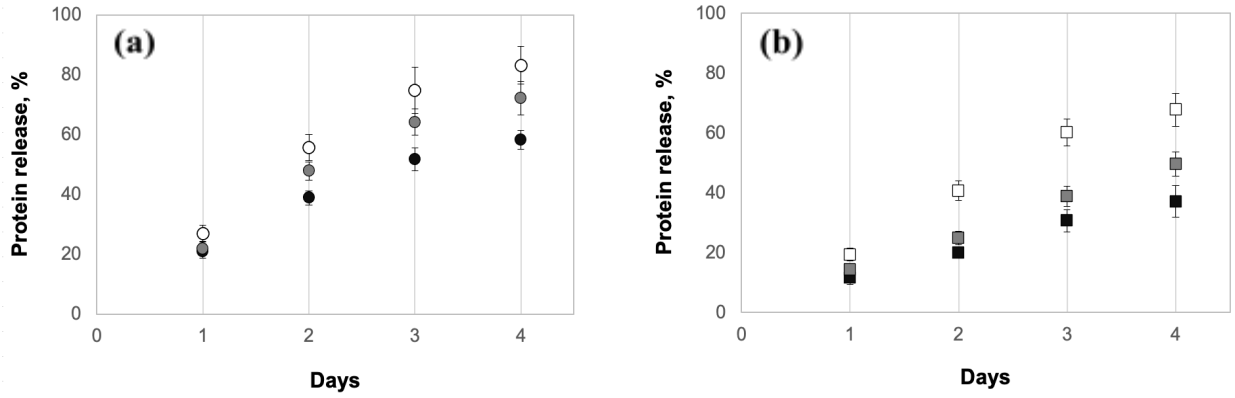

Figure 5. Double-network hydrogel results. Protein release from hydrogels prepared using 0% (white circles), 0.4% (gray circles), and 0.8% (black circles) chitosan for (**a**) 1% alginate and (**b**) 2% alginate. Data shown are the mean and standard error of triplicate measurements.

5. Discussion

5.1 Polymer Concentration Affects Protein Release

As shown in Figures 4 and 5, polymer concentration in both single and double network hydrogel drug delivery systems affected the release profiles of protein drugs. In both experiments as we increase the polymer concentration, we see a more linear release profile. For example, in Figure 4, the 1% alginate hydrogels appear to have a more logarithmic release profile. This nonlinear protein release profile is indicative of a burst release. In comparison, the 3% alginate hydrogel's release profile is much more linear with a lower slope, signifying a more controlled and stabilized release. This result is expected since a higher concentration of alginate allows for more crosslinking, which decreases the pore size of the hydrogel. Thus elevated levels of crosslinking within gels with higher polymer concentrations limit the amount of protein that can be released at once, discouraging burst releases. Overall, increasing the polymer concentrations within hydrogels encourages linear protein release profile, which is a valuable quality in drug delivery systems.

5.2 Controlling Drug Delivery with Double-Network Hydrogels

As shown in Figure 5, the addition of a second polymer also exhibited an effect on protein release rates. This is likely because the addition of a second polymer also increases the amount of crosslinking within hydrogel systems. Similarly, the experiments using double network hydrogels also exhibited the same trends in terms of changing polymer concentration. Furthermore, we noticed that increasing the alginate concentration, regardless of the chitosan, resulted in reduced release rates. These results were expected as our previous experiments on polymer concentration established this effect. Additionally, we can see in Figure 5 that increasing the concentration of chitosan in the DN hydrogels further reduced release rates indicating that the concentration of the individual polymers present in a DN hydrogel system has an effect on protein release rates.

These results also show that protein release rates can be further reduced through the use of double network hydrogels. The addition of a second network results in reduced release rates as the encapsulated protein not only has to get through one barrier, it also has to get through a second hydrogel barrier. A single network hydrogel only poses one barrier thus allowing protein molecules to pass more easily. The crosslinking of two hydrogel networks further reduces pore size as the pores of both the alginate and chitosan overlap. Moreover, the crosslinking density of the DN hydrogel system is increased due to the fact that both alginate and chitosan need to be crosslinked.

Finally, we saw that in our 1% alginate system, all of the experiments showed release profiles indicative of burst release. We also saw burst release in our 2% alginate system in the alginate hydrogels that were not crosslinked with chitosan. These results indicate that the addition of chitosan resulted in a more linear release profile. Thus, DN hydrogels offer yet

another method through which we can control and manipulate therapeutic protein delivery platforms using hydrogels.

6. Conclusion

6.1 Overall Findings

Through our research, we found two methods for controlling protein release in hydrogel systems. The first method is through varying hydrogel concentrations. In these experiments, we saw that increasing polymer concentrations decreases release rates. This shows that protein release rates may be manipulated by changing hydrogel concentrations. Furthermore, through our double-network hydrogel experiments, we found that the addition of a second hydrogel also reduces release rates. Within our double network system, we also found that the concentrations of the individual polymers affect protein release rates. These results show that double-network hydrogels provide an additional channel to control protein release rates. We believe our work will provide a foundation for future studies that aim to combine one or more properties of hydrogels to develop hydrogel-based drug delivery platforms for controlling the release of various therapeutic proteins.

6.2 Future Work

Future experiments aim to confirm the protein released from our hydrogels is active and can perform as expected. In these experiments, we will use horseradish peroxidase (HRP) as our model enzyme to test the viability and activity of protein within our system. We chose HRP because of its functionality and availability. HRP is an enzyme that catalyzes the oxidation of substrates using H_2O_2 as an oxidizing agent (Krainer, 2015). We will measure the HRP activity using another commercially available colorimetric assay, the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. This assay is often used to measure the concentration of antioxidants in samples (Ilyasov, 2020). Our preliminary experiments have confirmed that released proteins are stable within the hydrogel formulations and remain functional. Further experiments will confirm the percentage of activity retained as well as if the incorporation of protein within hydrogels leads to any structural changes.

Further work could be done with this project through the investigation of the effect of pH-responsive hydrogels on protein release rates. Through our literature research, we found that chitosan is a pH-responsive hydrogel. Experiments using chitosan hydrogels in varying pHs could provide insight into another potential mechanism through which therapeutic protein release rates could be controlled.

7. Engineering Standards and Realistic Constraints

Throughout the senior design process, we incorporated many engineering standards into the design of our hydrogel drug delivery system. Below, we discuss how these criteria impacted our design.

7.1 Health & Safety

Throughout our literature review, we ensured that all the hydrogels we were considering testing were nontoxic and biocompatible. Both alginate and chitosan possess these qualities ensuring that they would pose a low risk to patients in the future. If our product were to progress and be administered to patients, it would need to undergo many tests and trials to ensure that no damage is being harmed in the treatment process.

7.2 Ethics

Protein therapeutics possess the potential to act as a treatment for a variety of classes of diseases. This project works towards discovering a more safe and efficient method of protein drug delivery to better care for patients. The outcome of this project will hopefully indirectly improve the lives of patients by working towards more controlled methods of protein drug delivery. One ethical implication that is likely to come up as research within this field continues is animal testing. Fortunately, our project utilizes materials that have previously been shown to be biocompatible, so limited animal testing would be needed in the future. That being said, if this product is to have clinical uses in the future there will be tests that need to be conducted to ensure the safety of patients.

7.3 Manufacturability

When designing our project, our goal was to create a project that was not only safe and effective, but also inexpensive and simple. We tailored our project to be easily reproducible and intentionally used materials that were less expensive. Overall, the process did not take very long and the resources required were not very costly. Furthermore, the materials and assays that we used were commercially available,

7.4 Science, Technology, and Society

Since protein drugs utilize so many different mechanisms to cure disease, hydrogel-based drug delivery systems have the potential to help large quantities of people with different clinical processes. For example, data from this project could potentially be used to support the delivery of insulin via hydrogel systems, which would be a presumably safer and less invasive treatment. This would overall improve the quality of life of insulin-dependent patients by lowering the risk of complications with delivery. As mentioned before, our system is biocompatible, reproducible, and inexpensive. This presents the opportunity for more people to benefit from our project if developed into a viable drug delivery mechanism. This would increase accessibility to our product and treatment for plenty of diseases given what protein therapeutic is used.

7.5 Sustainability

One aspect of our project that we did not have enough time to explore is sustainability. It is unclear whether or not after being used our hydrogels are viable enough to be reused or recycled for future use as another product. It depends on what modes of drug delivery this project data is used for (oral, injectable, transdermal, etc). It is important to note, however, that hydrogels are biodegradable making them a more sustainable material for the development of drug delivery platforms.

8. Acknowledgments

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Appendix

Table 4. Project budget and purchased materials

Item	Vendor	Price
BCA Assay	Sigma Aldrich	$197.00
Sodium alginate	Sigma Aldrich	$143.00
Chitosan	Sigma Aldrich	$70.00
Human serum albumin (HSA)	Sigma Aldrich	$48.60
1 mL pipettes	Sigma Aldrich	$63.20
2 mL pipettes	Sigma Aldrich	$64.10
5 mL pipette	Sigma Aldrich	$65.90
10 mL pipette	Sigma Aldrich	$71.20
25 mL pipettes	Sigma Aldrich	$66.80
200 µl micropipette tips	Sigma Aldrich	$164.00
Magnetic stirrers - assorted sizes	Zoro	$47.75
1.5 mL Eppendorf tubes (500 count)	Sigma Aldrich	$55.20